B. Melanization of Nuclei, Chromidia and Mitoses.

VON SZILY, A. Ueber die Entstehung des Melanotischen Pigmentes im Auge der Wirbeltierembryonen und im Choroidealsarkomen. *Arch. Mikr. Anat.*, 77: 1-70, 1911.

"The colorless stromata of the melanin of metazoa originate in all cases examined exclusively in the nucleus. Their direct origin from the chromatin of the nucleus and their transformation into the cytoplasm can exactly be followed up. These 'chromidia' change over into melanin under the influence of ferments."

JELIASKOWA PASPALEWA, A. Cytologische Untersuchungen ueber die Entstehung des Melanotischen Pigments. *Ztschr. Wissenschaftl. Zoologie*, 137: 365-402, 1930.

"The observation of a total melanization of chromosomes makes it impossible to contest the origin of melanin from chromatin."

LUDFORD, R. J. Nuclear Activity during Melanosis with Special Reference to Melanin Formation in a Melanotic Sarcoma. *J. Royal Micr. Soc.*, 13-28, 1924.

———— The General and Experimental Cytology of Cancer. *J. Micr. Soc.*, 249-292, Sept. 1925.

"Melanosis occurring during mitosis and following chromatin extrusion, nuclear fragmentation, nuclear budding, karrhyorrhexis, and pycnosis." Ludford believes that the greatest part of the melanin is formed in the cytoplasm under the influence of the Golgi apparatus which is concerned with enzyme formation.

C. Melanization of Intranuclear Vacuoles.

MEIROWSKY, E. Die Entstehung des Oberhautpigments aus der Substanz der Kernkoerperchen. *Montsh. Prakt. Derm.*, 43: 155-163, 1906.

"A cavity is formed in the nucleus with melanin granules at its wall. Through the ruptured wall pigment is extruded into the protoplasm."

LUDFORD, R. J. Nuclear Activity During Melanosis with Special Reference to Melanin Formation in a Melanotic Sarcoma. *J. Roy. Micr. Soc.*, 13-28, 1924.

Summary 8. "Melanin is often formed inside the nucleus, generally in intranuclear vacuoles."

APITZ, K. Ueber die Pigmentbildung in den Zellkernen Melanotischer Geschwueslte. *Virchow Arch.*, 300: 89-112, 1937.

"The nucleus forms the mother substance of melanin." "The origin of the nuclear melanin takes place within vacuoles owing to the retention of physiological secretion product of the nucleus."

D. Biochemistry of Nucleoproteins.

DEROBERTIS, E. D. P., NOWINSKI, W. M. AND SAEZ, F. A. General Cytology. Publ. W. B. Saunders, Philadelphia and London, 1938.

E. Budding Processes in Individual Melanin Granules.

As far as known, no references are available.

Evidence for the Mitochrondrial Nature and Function of Melanin Granules.

MARK WOODS, HERMAN DUBUY & DEAN BURK.

National Institutes of Health, Bethesda, Md.

Melanin occurs in the cytoplasmic granules of the melanoblasts of the Harding-Passey and Cloudman S91 mouse melanomas, and also, of course, in the phagocytes of these tumors. In both tumors, cells occur in which all, or nearly all, of the visible cytoplasmic granules are melanized. Colorless granules of similar size occur in amelanotic or partially amelanotic cells of the S91 tumor and also in the derived Algire partially amelanotic S91A melanoma. Some colorless granules usually occur in the perikaryon of the Harding-Passey melanoblast. The S91A amelanotic melanoma sometimes contains cells in which the cytoplasmic granules are very slightly melanized.

The cytoplasmic granules (melanized and non-melanized) are the only structures which stain with Janus Green B in the absence of nuclear staining by this dye. The granule staining is reversibly dependent upon oxygen tension. No other structures resembling mitochondria are visible in the cells. On alkaline hydrolysis, both melanized and non-melanized granules yield solutions with strong ultraviolet absorption at 2580A, and contain organic phosphorus and pentose. Centrifugally isolated cytoplasmic granules of amelanotic melanoma cells possess enzymic activities characteristic of mitochondria of other origin (e.g., from liver, kidney, heart). Aerobically, these activities include the cytochrome oxidase and succinic oxidase systems. Anaerobically, the cytoplasmic granules possess glycolytic activities also comparable to those of typical mitochondria. Centrifugally isolated melanized granules from both Harding-Passey and S91 melanomas possess not only all of the enzymic activities found in the amelanotic granules, but also dopa oxidase activity. On the basis of the foregoing morphologic, chemical and enzymic data it is concluded that the melanized and non-melanized granules of these mouse melanomas are mitochondria.

Mitochondria are fundamental structures in both animal and plant cells, and in plants it is now well established that they derive from pre-existing mitochondria, possess a complex hereditary system (chondriogenes), and are capable of mutation. In mutant states plant mitochondria display abnormal enzymic activities. Self-duplication of these abnormal mitochondria results in development of neoplasia. Plant mitochondria may be specifically modified by certain viruses that result in neoplasias very similar to those caused by mutant chondriogenes. The behavior of normal, mutant or virus-modified mitochondria may also be affected by specific nuclear genes.

BIBLIOGRAPHY.

1. WOODS, M. W., DUBUY, H. G., BURK, DEAN AND HESSELBACH, M. L. Cytological studies on the nature of the cytoplasmic particulates in the Cloudman S91 mouse melanoma, *J. N. C. Inst.*, 9: 311-323, 1949.
2. DUBUY, H. G., WOODS, M. W., BURK, DEAN AND LACKEY, MARY D. Enzymatic activities of isolated amelanotic and melanotic granules of mouse melanomas and a suggested relationship to mitochondria. *Ibid.*, 9: 325-336, 1949.
3. DUBUY, H. G. AND WOODS, M. W. A possible common mitochondrial origin of the variegational and virus diseases in plants and cancers in animals. In Am. Assoc. Advancement Sc. Res. Conf. on Cancer, pp. 162-169. Lancaster press (1945).
4. WOODS, M. W. AND DUBUY, H. G. Cytoplasmic diseases and cancer. *Science*, 102: 591-593, 1945.

Oxidative Activities of Mouse Melanomas with Reference to Melanization.

MARIE L. HESSELBACH, MARK WOODS & DEAN BURK.

National Institutes of Health, Bethesda, Md.

Dopa Oxidase. In the absence of added cytochrome C, Harding-Passey mouse melanoma homogenates oxidized tyrosine, dopa and catechol, with consumption of oxygen gas and formation of dark brown or black coloration. Cloudman S91 melanoma homogenates acted likewise on dopa and catechol. Tyrosine was oxidized either not at all or following a variable lag period. Similarly prepared homogenates of the derived Algire partially amelanotic melanoma S91A did not measurably oxidize any of the phenols or produce browning, nor did they retard the oxidation of dopa by S91 homogenates to which they were added. Normal liver homogenates behaved like the S91A extracts.

Cytochrome Oxidase. In the presence of added cytochrome C, oxidation of dopa was markedly increased in the S91 melanoma homogenates, and also now occurred extensively in the S91A amelanotic melanoma and liver homogenates. This second type of dopa oxidation took place via cytochrome oxidase, not only because cytochrome C was required but because the action could be eliminated entirely by pretreatment of the homogenates with 70% ethyl alcohol, a treatment that did not decrease true dopa oxidase activity, in fact, often enhanced it, suggestive of elimination of a dopa oxidase inhibition. In no instance was tyrosine oxidized by the cytochrome system.

A third type of melanization was observed in S91AB derivatives of S91A tumors that were obtained by prolonged transfer of the latter in brown dba mice instead of white C mice. Such tumors eventually became highly pigmented, but their homogenates at this stage oxidized dopa only in the presence of added cytochrome C. However, homogenates of later transfers gradually came to show, without added cytochrome C, endogenous dopa oxidation enhanced by the alcohol treatment, indicating eventual development of true dopa oxidase activity in addition to oxidation via cytochrome oxidase. None of the amelanotic tumors, or their secondarily melanized derivatives, oxidized tyrosine except the S91AB tumor that had undergone prolonged passage (35 generations) in dba mice. In this instance oxidation was not manometrically detectable within the first 4 hours although the extracts to which tyrosine had been added blackened after ca. 24 hours.

Even though the S91AB tumor, maintained by prolonged transplantation in dba mice, assumed the enzymic pattern of the original metastatic S91 tumor, it remained biologically distinct. Thus like the original S91A amelanotic tumor, from which it was derived, it gave no evidence of producing metastases.

BIBLIOGRAPHY.

1. WOODS, M. W., DUBUY, H. G., BURK, DEAN AND HESSELBACH, MARIE. Cytological studies on the nature of the cytoplasmic particulates in the Cloudman S91 mouse melanoma. *J. N. C. Inst.*, 9: 311-323, 1949.
2. DUBUY, H. G., WOODS, M. W., BURK, DEAN AND LACKEY, MARY D. Enzymatic activities of isolated amelanotic and melanotic granules of mouse melanomas and a suggested relationship to mitochondria. *Ibid.*, 9: 325-336, 1949.
3. BURK, DEAN, ALGIRE, G. H., HESSELBACH, MARIE L., FISCHER, CLARA E. AND LEGALLAIS, FRANCES Y. Tissue metabolism of transplanted mouse melanomas, with special reference to characterization by paraphenylenediamine. Special Publications of the New York Academy of Sciences, IV, 437-446, 1948.
4. HESSELBACH, MARIE L. Cytochemical studies of certain melanotic and amelanotic mouse tumors. Master's thesis. Dept. of Chemistry, Georgetown University, Washington, D. C. May, 1949.